DDT and the Frequency of Implanted Ova in the Mouse

by C. LUNDBERG and J. E. KIHLSTRÖM
Institute of Zoophysiology
Box 560
S-75122 Uppsala, Sweden

Like many other substances chlorinated hydrocarbons,eg. DDT, stimulate the synthesis of microsomal enzymes in the liver of the rat (MORELLO 1965,CONNEY 1967), thereby increasing the metabolism of drugs. In the mouse, however, HART and FOUTS (1965) found no induction of the microsomal enzymes after DDT-treatment. Later CRAM and FOUTS (1967) reported that DDT could also stimulate drug-metabolism in the mouse but that it was less potent than in the rat. Also ABERNATHY et al. (1971) showed that there was an increased enzyme activity in the mouse caused by DDT. It thus seems that differences between both species and breeds can explain the divergency of the obtained results.

Many research workers (KUNTZMAN et al.1965, PEAKALL 1967, RISEBROUGH et al.1965) have shown that there is a correlation between increased enzyme activity caused by chlorinated hydrocarbons and an enhanced steroid breakdown. Administration of DDT therefore may cause changes in the levels of steroid hormones possibly resulting in a changed reproductive capacity in the individuals. The purpose of the present investigation was to study the effects of injections of DDT on the number of implanted ova in the mouse.

Material and Methods

About 270 sexually mature female mice weighing 18.0-38.5 g, age 40-60 days, and about 100 males (the NMRI strain) have been used for the experiments. In each experimental group and its corresponding control all animals were of the same age, and the animals were distributed between the experimental and control groups as equally as possible with regard to their body weights. After mating the females were housed separately and given pellets (Standard food for rats and mice,Ewos Ltd.) in excess once a day. The day of occurrence of the vaginal plug was designated as day 1 of pregnancy. DDT was administered by intraperitoneal injections at different occasions after mating (see the Tables). This route of administration was chosen in order to avoid the eventual breakdown of DDT by the microflora in the intestine (BARKER et al.1965, MENDEL and WALTO 1966). The studied doses were

Bulletin of Environmental Contamination & Toxicology,
Vol. 9, No. 5

TABLE I

Implantation of ova in the mouse after administration of DDT day 1 of pregnancy. Dissection of the animals about day 12 of pregnancy.

Dose, mg/kg	Number of animals	Number of corpora lutea	Number of implantations	Per cent of ova implanted	x^2	p <
A. All mated females.						
20 contr.	35	410	332	81	0.91	0.40
exp.	31	391	305	78		
50 contr.	29	369	262	71	17.1	0.0005
exp.	28	354	196	55		
100 contr.	18	219	131	60	22.6	0.0005
exp.	21	262	106	41		
B. Only females with implantation sites.						
20 contr.	31	375	332	89	0.73	0.40
exp.	27	336	305	91		
50 contr.	23	299	262	88	8.5	0.005
exp.	19	254	196	77		
100 contr.	12	145	131	90	18.7	0.0005
exp.	12	156	106	68		

20, 50 and 100 mg/kg body weight. The control animals were given the corresponding volume of the solvent, arachis oil, 0,01 ml/g body weight. The purity of the preparation of p,p' - DDT used (1,1-Bis-(4-chlorphenyl)-2,2,2-trichlorethan,Fluka Ag,Switzerland) was stated as purissimum. Gas chromatographic analyses of the preparation show that o,p' - DDT and p,p' - DDD are present in small amounts. The animals were killed on day 10-15 of pregnancy. The number of animals per group is given in Tables I-II. After dissection the numbers of corpora lutea, placentae and embryos were counted, considering each placenta with or without an embryo as indicating an implantation. The developmental stages of the corresponding fetuses were also registered. Apparently, a few non-pregnant mice had not ovulated as no corpora lutea could be detected. These animals were therefore excluded. In animals without implantations there were sometimes difficulties in counting the number of corpora lutea. Therefore the results are presented in two ways: the one (A) including all mated females, the other (B) only those with at least one implantation site. The dissector was unaware wether the dissected animal was an experimental or a control one. The figures obtained have been analysed using a chi-square test for heterogenity.

Results and Discussion

The results are presented in Tables I and II. As seen in Table I (administration of DDT day 1 of pregnancy) there is a significant decrease in the number of implanted ova at doses of 50 and 100 mg/kg body weight, compared with the controls, while a dose of 20 mg/kg causes no significant decrease. However, when DDT is administered both day 1 and day 3 at a dose of 20 mg/kg per injection the decrease in the number of implanted ova is highly significant (Table II).

Though the number of non-pregnant females in the experimental series is slightly higher than that in the control series, there is no statistically significant difference in the number of non-pregnant females in any of the experimental series. No differencies with regard to the developmental stage of the fetuses could be detected at the dissection. Gas chromatographic analyses show that DDE, p,p' - DDD and p,p'- DDT are present in the livers of the experimental animals in amounts not exceeding those found in wild life (DIMOND and SHERBURNE 1969) but no further quantifications were made.

The observed decrease in the frequency of implanted ova may be due to an alteration of the hormonal balance in the body. In ovariectomized mice progesterone alone does not initiate implantation, but a single injection of estrogen on day 4, 5 or 6 in combination with progesterone treatment gives a nearly normal frequency of implantation (SMITH 1968). An enhanced hydroxilating enzyme activity may therefore cause a lowering of the estrogen amount below the level necessary for a successful implantation.

TABLE II

Implantation of ova in the mouse after administration of DDT both day 1 and day 3. Dissection of the animals about day 12 of pregnancy.

Dose, mg/kg	Number of animals	Number of corpora lutea	Number of implantations	Per cent of ova implanted	x^2	p <
A. All mated females.						
contr.	31	383	296	77		
20					32.2	0.0005
exp.	28	347	199	57		
B. Only females with implantation sites.						
contr.	25	338	296	88		
20					14.26	0.005
exp.	20	264	199	75		

This hypothesis may explain the present results. However further experiments in order to study the mechanism behind the obtained DDT - effects are necessary before any definite conclusions can be made.

Acknowledgement

Miss E-B. Norlin, Miss M. Larsson and Mr. I. Kihlström are gratefully acknowledged for technical assistance. This work was supported by the National Swedish Environment Protection Board and the Faculty of Mathematics and Science at the University of Uppsala.

References

ABERNATHY, C.O., HODGSON, E. and GUTHRIE, F.E.: Biochem. Phamacol 20, 2385 (1971).
BARKER, P.S., MORRISON, F.O. and WHITAKER, R.S.: Nature 205, 621 (1965).
CONNEY, A.H.: Pharmacol. Rev. 19, 317 (1967).
CRAM, R.L. and FOUTS, J.R.: Biochem. Pharmacol. 16, 1001 (1967).
DIMOND, J.B. and SHERBURNE, J.A.: Nature 221, 486 (1969).
HART, L.G. and FOUTS, J.R.: Naunyn-Schmiedeberg's Arch. exp. Pathol. u. Pharmakol. 249, 486 (1965).
KUNTZMAN, R., WELCH, R.M. and CONNEY, A.H.: Adv. Enzyme Reg. 4, 149 (1965).
MENDEL, J.L. and WALTON, M.S.: Science 151, 1527 (1966).
MORELLO, A.: Can. J. Biochem. and Physiol. 43, 1289 (1965).
PEAKALL, D.B.: Nature 216, 505 (1967).
RISEBROUGH, R.W., RIECHE, P., PEAKALL, D.B., HERMAN, S.G. and KIRVEN, M.N.: Nature 220, 1098 (1968).
SMITH, D.M.: J. Endocrinol. 41, 11 (1968).